IMMUNOHISTOCHEMICAL ASSESSMENT OF ULCERATIVE COLITIS-RELATED DYSPLASIA

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Summary

In longstanding ulcerative colitis (UC) it is difficult to detect dysplasia endoscopically and to discriminate these changes from inflammatory regenerative epithelium pathologically. To detect UC associated dysplasia at an earlier stage and to distinguish regenerative changes from premalignant ones. In 78 UC cases showing features of high-grade dysplasia (n = 16), low-grade dysplasia (n = 24), 'indefinite for dysplasia' (n = 14), or regenerative atypia (n = 24) we studied location and intensity of Ki67 and p53 to detect differences in the frequency and pattern of nuclei positive for the proliferation markers. Regarding to Ki67 staining, the results were divided into 4 categories: 'basal zone' (staining restricted to the basal third of the crypt); 'mid-zone' (extension into the middle third); 'top zone' (extension into the upper third); and 'surface' (extension into the surface epithelium). Related to p53 immuno-staining we assessed: location and intensity (weak, moderate, strong).

In high grade dysplasia the distribution of Ki-67 positive cells was diffuse throughout the full length of the crypt, whereas low grade dysplasia and epithelium indefinite for dysplasia, as well as regenerative epithelium, showed an expanded basal zone. The rate of p53 overexpression was significantly higher in UC associated colorectal cancer than in non-neoplastic mucosa. None of the regenerative atypia cases showed strong intensity p53 staining compared to dysplasia cases. Assessment of Ki-67 and p53 immunostaining could be combined with routine histological evaluation in longstanding UC to improve the diagnostic accuracy and to appreciate the risk of malignant transformation.

Key words: ulcerative colitis, immunohistochemistry, dysplasia, colorectal carcinoma

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Introduction

Colorectal carcinoma is a dreadful complication of UC. The risk of developing cancer increases in patients with longstanding UC (7 to 10 years) with a rate of approximately 0.5-1% per year. Because of the fact that during the last period of time the incidence of colorectal cancer became higher among patients with UC we can say that routine colonoscopy becomes compulsory (Torres and Ríos, 2008). However dysplastic lesions are difficult to recognize by means of endoscopy. It is also a tough task to differentiate them from regenerative changes of the epithelium. So, it becomes clear that we need some additional methods to improve the early detection of cancer.

Epithelial dysplasia in UC is divided into low grade dysplasia and high grade dysplasia depending on the severity of morphological changes. Low grade dysplasia needs careful colonoscopic following whereas high grade dysplasia is a clear indication for colectomy, so, the risk of imminent development of cancer can be eliminated (Shigehiko et al., 2008).

Evaluation of dysplasia in longstanding UC is a difficult and often subjective task. Although the routine pathological examination gives important information about the chronic
inflammation-carcinoma sequence, the relationship between the morphological changes or remodeling of regenerated mucosa and carcinoma development is yet to be clarified.

Histopathological examination using hematoxilin-eosin staining is still a standard method in grading dysplasia and assessment of regenerative tissue changes although we feel the need of some additional analysis methods to increase the accuracy and precocity of the diagnosis.

Ki-67 is a huge nuclear protein which plays an important role in cellular proliferation. The monoclonal antibody Ki-67 detects a nuclear antigen that reflects cell proliferation, thus identifying the growth fraction of tissues and tumors (Suzuki et al., 1992).

Recently, Ki-67 like antibodies (MIB 1) were developed that are reactive in sections of formalin fixed, paraffin wax embedded tissue after antigen retrieval.

P53 is a protein which plays an important role in regulating cell cycle and in controlling the cellular proliferation. A mutation in this protein is the most common finding in human cancers (Harpaz et al., 1994).

The purpose of our paper was to detect UC associated dysplasia at an earlier stage and to distinguish regenerative changes from premalignant ones.

**Material and methods**

We studied 78 people with longstanding UC (> 8 years): 16 high-grade dysplasia, 24 low-grade dysplasia, 14 indefinite for dysplasia, 24 regenerative atypia cases.

We studied location and intensity of Ki67 and p53 to detect differences in the frequency and pattern of nuclei positive for the proliferation markers in 156 sections from paraffin embedded biopsies.

The biopsies obtained during colonoscopy, after specific preparation were cut into 3-4 µ thick sections (8-10 per case). In order to establish the histopathological diagnosis and to include the case into a group of lesions, the first sections were stained using routine H&E histological methods using a light microscope Carl Zeiss Ergaval.

Then the biopsy specimens were selected and then stained immunohistochemically to detect differences in the frequency and pattern of nuclei positive for the proliferation marker Ki-67 and p53.

For Ki67 immunostaining, antigen retrieval was carried out and then the sections were stained using the MIB-1 antibody to Ki67 at a dilution of 1:100, using Dako ChemMate™ reagents and detection agents as described in the manufacturer’s operating manual.

For p53 immunoassaying, antigen retrieval was carried out using citric acid buffer (1.05 g in 500 ml distilled water, pH 6) and microwaving at full power for 20 minutes. Sections were pretreated with 1.5% hydrogen peroxide for 15 minutes followed, after antigen retrieval, by 1:5 normal rabbit serum for 10 minutes and then the DO-7 antibody to p53 (Dako, UK) at a dilution of 1:100 for 60 minutes. Visualization was achieved by incubation with rabbit anti-mouse antibody (1:400) for 30 minutes.

A single observer assessed all sections blindly.

Cases were divided into four groups depending on the extent of Ki67 staining:

- 'basal zone' (staining restricted to the basal third of the crypt)
- 'mid zone' (extension of staining into the middle third)
- 'top zone' (extension into the upper third) –fig. 3;
- 'surface' (extension into the surface epithelium).

The evaluation of p53 immunoassaying was carried out using a semiquantitative method:

- positive p53 immunoreaction- >5% stained nuclei
- negative p53 immunoreaction- <5% stained nuclei.
Results

In low grade dysplasia, most of the cases had middle and top localization of the staining.

Fig. 1. Extension of staining into the middle third of the crypts. Ki67 immunostaining, DAB.

In high grade dysplasia we did not identify any case showing basal Ki67 immunostaining. We found to be characteristic for high grade dysplasia the top and surface staining of the crypts.

Fig. 2. Low grade dysplasia. P53 immunostaining, DAB.

In regenerative atypia cases, we observed the predominance of restricted Ki67 staining to the basal third of the crypt (50% - 12 cases).

All regenerative atypia cases were p53 negative.

Difficult cases, interpreted as “indefinite for dysplasia” were p53 negative in 86% of the cases.

Four “indefinite for dysplasia” UC which expressed ki-67 surface staining were proved to be p53 positive so they were readmitted in dysplastic UC.

In adenocarcinoma cases the immunoassay showed a completely diffuse aspect.

Fig. 3. High grade dysplasia. Ki-67 immuno staining, DAB.

Fig. 4. High grade dysplasia. P53 immunostaining, DAB.

Fig. 5. G2 adenocarcinoma with positive p53 and diffuse staining pattern of Ki-67 (>90%). DAB.
Discussion

We have investigated whether Ki67 and p53 immunostaining can be used to aid the diagnosis and grading of UC-related dysplasia.

It can be difficult to separate the benign regenerative atypia of the epithelium from true dysplasia, using histological criteria alone. It is also difficult then to assess the grade of dysplasia once confirmed. Ki67 and p53 expression may offer an objective mean of doing this.

Our data refute the previously published suggestion that 'diffuse' Ki67 staining is a specific sign of dysplasia (Noffsinger et al., 1996; Bierla et al., 1998; Andersen et al., 1998). Increased cell proliferation is a well recognized finding in areas of healing and active colitis (Bleiberg et al., 1980). While Noffsinger and colleagues stated that some of their nondysplastic cases showed 'foci of active inflammation' and found focal surface epithelial staining in three of their nondysplastic cases, (Noffsinger et al., 1996) no comment was made as to whether the two were related. We have confirmed the findings previous studies that restriction of staining to the basal third of the crypt is not seen in cases of UC-related dysplasia. However, this observation of restricted basal third Ki67 staining did suggest that a third of our 'indefinite for dysplasia' cases might be reassigned to the regenerative atypia category.

P53 overexpression in nondysplastic mucosa has been reported in at least two previous studies (Krishna et al., 1995; Lashner et al., 1999). Most p53 studies in ulcerative colitis have shown that the frequency of immunohistochemical overexpression and point mutation increases with the severity of dysplasia and this suggests that it is probably a late event (Taylor et al., 1993; Zivković et al., 2007; Noffsinger et al., 1996, Kullmann et al., 1996).

One important function of p53 lies in its role in the induction of apoptosis. The reduction in the ability to undergo apoptosis may lead to immortalization of cells. This would tend to increase the accrual of mutations, which may eventually be sufficient for malignant transformation (Ilyas and Talbot, 1995).

The negative predictive value of p53 staining for excluding a diagnosis of high grade dysplasia is twice that of Ki67 staining but not much lower than that of the two tests combined (Wong et al., 2000). We emphasize that p53 immunostaining is a good marker for assessing genetic alterations that precede histologically malignant change and for diagnosing carcinoma in UC or other colorectal diseases. In the same time, given the high degree of sensitivity of antigen retrieval methods, in our study we might have detected overexpression of wild type protein - that is, appropriate expression of non-mutant p53 - in an attempt to induce apoptosis in neoplastic cells.

Little attention has been paid to developing a tool which may aid the differentiation between low and high-grade UC-related dysplasia. This may be attributable to the argument that LGD
should be managed in the same way as HGD, a viewpoint discussed in Harpaz and Talbot's recent review (Harpaz and Talbot, 1996).

We have demonstrated that restriction of Ki67 or p53 staining to the lower two-thirds of the crypt excludes a diagnosis of HGD.

A new study with a larger patient population might show whether Ki-67 and p53 based classification of mucosal alterations related to longstanding ulcerative colitis is a more objective way of predicting malignant transformation than traditional histopathological evaluation alone.

Conclusions

The Ki-67 immunostaining extended to the top of the glandular crypts and along the surface epithelium represents an important clue for the existence of ulcerative colitis related dysplasia.

Restriction of Ki-67 staining to the basal third of the crypt appears to exclude a diagnosis of dysplasia.

P53 positivity in LGD and HGD shows that the mutation of p53 gene is an early event in colorectal carcinogenesis.

Intense immunostaining for p53 is an important diagnostic tool in UC associated dysplasia.

Assessment of Ki-67 and p53 immunostaining could be combined with routine histological evaluation in longstanding UC to improve the diagnostic accuracy and to appreciate the risk of malignant transformation.

References


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